5.0 Test and calibration methods and method validation

Each laboratory shall have a quality system. The laboratory's Quality System is the process by which the laboratory conducts its activities so as to provide the client with data of known and documented quality with which to demonstrate regulatory compliance and for other decision-making purposes. This includes how analytical methods are selected, evaluated, and employed. The quality system includes the quality assurance (QA) policies and quality control (QC) procedures. The quality system shall be documented in the laboratory's quality manual.

This chapter contains detailed quality system requirements for consistent and uniform implementation by both the laboratories conducting testing under these standards and the evaluation of those laboratories by accrediting authorities. Each laboratory seeking accreditation under NELAP must assure that they are implementing their quality manual and that all the QC procedures specified in this Chapter are being followed. The QA policies, which establish essential QC procedures, are applicable to environmental laboratories regardless of size and complexity.

All items identified in this Chapter shall be available for on-site assessment or data audit

5.5.4.1 Standard Operating Procedures and Laboratory Manual(s)

The laboratory shall maintain a methods manual. The methods manual shall contain the laboratory's standard operating procedures (SOP's). The SOP's shall accurately reflect all phases of current laboratory activities such as sample receipt, sample storage, sample analysis, assessing data integrity, corrective actions, handling customer complaints, all test methods, and data and record storage. All confidential business information in the methods manual shall be marked by the laboratory "CBI".

a) An SOP may be an equipment manual provided by a manufacturer, or an internally written document so long as the SOP is adequately detailed to permit someone other than the analyst to reproduce the procedures that had been used to produce a given result.

b) The test method SOP's may be copies of published methods as long as any changes or selected options in the methods are documented and included in the SOP's (see below). Reference test methods that contain sufficient and concise information on how to perform the tests do not need to be supplemented or rewritten as internal procedures if these methods are written in a way that they can be used as published by the laboratory. It may be necessary to provide additional documentation for optional steps in the method or additional details.

c) Copies of all SOP's shall be accessible to all appropriate personnel.

d) SOP's shall be organized in a manner such that they are easily accessible to an assessor.

e) Each SOP shall clearly indicate its effective date, its revision identifier, and shall bear the signature(s) of the approving authority.

f) Each test method SOP shall give or reference the following information, where applicable:

1.0 Scope and Application
2.0 Summary of Method
3.0 Definitions
4.0 Interferences
5.0 Safety
6.0 Equipment and Supplies
7.0 Reagents and Standards
8.0 Sample Collection, Preservation, and Storage
9.0 Quality Control
10.0 Calibration and Standardization
11.0 Procedure
12.0 Data Analysis and Calculations
13.0 Method Performance
14.0 Pollution Prevention
15.0 Waste Management
16.0 References
17.0 Tables, Diagrams, Flowcharts, and Validation Data

5.5.4.2 Selection of methods

The laboratory shall utilize methods within its scope (including sample collection, sample handling, transport and storage, sample preparation and sample analysis) which are appropriate and applicable to client needs (i.e., to meet regulatory or other requirements specified by the client). These requirements may specify that a particular method or group of methods be employed for a given project or program, or that specific data or measurement quality objectives be achieved, or both (i.e., data or measurement quality objectives specified by the client or required of the client to demonstrate regulatory compliance define the boundary conditions of the method selection process).

When the use of a particular test method is mandated by regulation or is requested by a client, only that method shall be used. Deviations from a reference test method shall occur only if the deviation has been documented, technically justified, authorized, and accepted by the client. The laboratory shall inform the client when the method proposed by the client is considered not capable of providing data consistent with intended use or out of date and the communication shall be documented. Client approval of the methods to be used when conducting analyses must be obtained prior to implementation. Modifications must be documented in and referenced in reports to the

client.

When the client does not specify the method to be used, the laboratory shall select methods that are appropriate for the intended use. Such methods may be those published in international, regional, or national standards, or by reputable technical organizations, or in relevant scientific texts or journals, or as specified by the manufacturer of the equipment, or laboratory-developed methods or methods adapted by the laboratory.

All measurements made while operating as a NELAC accredited laboratory must have an adequate demonstration that the measurement system provided data consistent with its intended use. The laboratory shall ensure the quality of results provided to clients by implementing a system to document the quality of the laboratory's analytical results.

This demonstration consists of four activities:

1) an initial determination that the measurement system is capable of providing data of the quality needed to meet client and/or regulatory requirements (see Appendix C);

2) an acceptable instrument calibration and verification that the system has remained calibrated during the period that it was used for analysis;

3) documentation that the laboratory is operating with all analytical systems functioning within the quality assurance and quality control protocols and procedures described in their quality system documents (see Appendix D) and;

4) documentation of the quality of any data that was obtained. The specific activities performed for this demonstration are defined below and in Appendices C and D.

5.5.4.3 Not needed

5.5.4.4 Not needed

5.5.4.5 Validation of methods

The laboratory must routinely evaluate and document the quality of the measurement system relative to the materials being tested. This activity is termed "method evaluation." The thoroughness and robustness of the evaluation depends on what is already known about the performance of the method on the analyte-matrix combination of concern over the concentration range of interest. Properties of the measurement system to be evaluated include bias, precision, sensitivity, and selectivity. The measurement system includes the analyst (operator) or work cell and method.

Essential elements of method evaluation include measures to determine accuracy (i.e., systematic

positive or negative bias, and precision - random error), to determine if the system has adequate sensitivity, the range of measurement capability, and verification of adequate system selectivity for the intended purpose.

The laboratory shall evaluate each method for its intended use according to Appendix C. The laboratory shall record the results of the evaluation, the protocol used for the evaluation, and the basis for the stated measurement system performance. When changes are made in a method, the influence of such changes shall be documented and, if appropriate, a new evaluation shall be carried out.

The thoroughness of any method evaluation is always a balance between costs, technical possibilities, available time, and the consequences of error. There are many cases in which the range and uncertainty of the values (e.g., accuracy, detection limit, selectivity, linearity, repeatability, reproducibility, robustness and cross-sensitivity) can only be approximated. However, so long as the level of approximation is commensurate with the needs of the client, such tradeoffs are acceptable.

5.5.5.2 Calibration

Calibration requirements are divided into two parts: (1) requirements for analytical support equipment, and 2) requirements for instrument calibration. In addition, the requirements for instrument calibration are divided into initial instrument calibration and continuing instrument calibration verification.

5.5.5.2.1 Support Equipment

These standards apply to all devices that may not be the actual test instrument but are necessary to support laboratory operations. These include but are not limited to: balances, ovens, refrigerators, freezers, incubators, water baths, temperature measuring devices (including thermometers and thermistors), thermal/pressure sample preparation devices, and volumetric dispensing devices (such as Eppendorf or automatic dilutor/dispensing devices) if quantitative results are dependent on their accuracy, as in standard preparation and dispensing or dilution into a specified volume.

(a) All support equipment shall be maintained in proper working order. The records of all repair and maintenance activities including service calls, shall be kept.

(b) All support equipment shall be calibrated or verified at least annually, using NIST traceable references when available, over the entire range of use. The results of such calibration shall be within the specifications required of the application for which this equipment is used or:

(1) The equipment shall be removed from service until repaired; or

(2) The laboratory shall maintain records of established correction factors to correct all measurements.

(c) Raw data records shall be retained to document equipment performance.

(d) Prior to use on each working day, balances, ovens, refrigerators, freezers, and water baths shall be checked in the expected use range, with NIST traceable references where available. The acceptability for use or continued use shall be according to the needs of the analysis or application for which the equipment is being used.

(e) Mechanical volumetric dispensing devices including burettes (except Class A glassware) shall be checked for accuracy on at least a quarterly use basis. Glass microliter syringes are to be considered in the same manner as Class A glassware but must come with a certificate attesting to established accuracy, or the accuracy must be initially demonstrated and documented by the laboratory.

(f) For chemical tests the temperature, cycle time, and pressure of each run of autoclaves must be documented by the use of appropriate chemical indicators or temperature recorders and pressure gauges.

(g) For biological tests that employ autoclave sterilization, see Section D.3.8.

5.5.5.2.2 Instrument Calibration

This standard defines the requirements that laboratories must follow to ensure that all instruments used for analysis are properly calibrated before and during their use in order for the data to be of known quality and appropriate for the intended use. This standard does not specify detailed procedural steps ("how to") for calibration but establishes the minimum essential elements. This approach allows flexibility and permits the employment of a wide variety of analytical procedures and statistical approaches. At a minimum these essential elements must be addressed during spectrochemical, electrochemical, and chromatographic test procedures. Other sections in this chapter address the essential elements for additional test procedures, such as Appendix D.4.4 for radiochemistry. If more stringent standards or requirements are included in the particular test method being used for analysis, the method standards shall apply and the laboratory demonstrate that such standards are met. If it is not apparent which standard is more stringent, then the requirements of the method or regulation are to be followed.

Note: In the following sections, initial instrument calibration (calibration) is directly used for quantitation and calibration verification is used to confirm the continued validity of the initial calibration.

5.5.5.2.2.1 Initial Calibration

The following items are essential elements of calibration:

a) All instruments shall be calibrated before use and maintained in a calibrated state during use.

b) The calibration shall be verified with a standard(s) prepared independent of the standards used for calibration. This verification shall be performed with a standard obtained from a second manufacturer, or it can be a second standard obtained from the manufacturer if the second lot can be demonstrated to have been prepared independently from the first lot.

c) The details of the calibration procedures including calculations, integrations, acceptance criteria and associated statistics shall be included or referenced in the test method SOP. When calibration procedures have been specified in a referenced test method, then a copy of the referenced method must be retained by the laboratory and be available for review. When the calibration procedure is specified by regulation or by the client (including the number of calibration points), calibration shall be performed as specified.

d) Results of the calibration must be documented and retained by the laboratory and be available for review. Sufficient raw data records must be retained to permit reconstruction of the calibration (e.g., calibration date, test method SOP, instrument identifier, each analyte name, analyst's name; concentrations used, and instrument responses obtained, calibration line or curve or response factor, or unique equation or coefficient used to convert analytical system responses to concentrations or amounts).

e) All sample results shall be quantitated from the calibration and shall not be quantitated from any calibration verification, unless specifically required by the method or client.

f) Calibrations shall be traceable to a national standard, when available. The lower calibration standard shall be at or below the lower quantitation limit and the upper calibration standard at or above the upper quantitation limit (see Appendix C).

g) Measured concentrations that are outside of the instrument calibration range shall be reported as having less certainty (e.g., defined qualifiers or flags or explained in the case narrative). The lowest demonstrated quantitation limit is the lowest concentration that data shall be reported with certainty.

h) If the calibration results are outside established acceptance criteria, corrective actions must be performed. Data associated with an unacceptable initial instrument calibration shall not be reported without qualifiers and explanation.

i) Criteria for the acceptance of calibration shall be established (e.g., correlation coefficient or relative standard deviation of calibration or response factors). The criteria used must be appropriate to the calibration technique employed.

j) If the method being employed for the analysis does not specify the number of calibration points, the minimum number must reflect the objectives of the analysis and the linearity of the instrument. For example, if the data only needs to show that a result is above or below a certain number (e.g., a regulatory limit), a single point calibration at that limit is sufficient. A single point standard at the reporting limit is also sufficient to demonstrate absence of the analyte. For detectors with a very linear response, a calibration line between a single point and a blank or zero may be sufficient. In this case, the sensitivity, linearity, and accuracy must be demonstrated by quantitation of known standards at the low, mid, and high points of the calibration. The laboratory must have a standard operating procedure for determining the number of points for establishing the initial instrument calibration.

5.5.5.10 Calibration Verification

When the initial instrument calibration is not performed on the day of analysis, the validity of the instrument calibration shall be verified prior to sample analyses by a calibration verification with each analytical batch. Calibration shall be verified before conducting any analyses and at the end of each analytical batch. The following items are essential elements of calibration verification:

a) The details of the calibration verification procedure, calculations, and associated statistics must be included or referenced in the test method SOP.

b) Calibration shall be verified for each compound, element, or other discrete chemical species, except for mixtures such as Aroclor-1254, Total Petroleum Hydrocarbons, or Toxaphene where a representative chemical related substance or mixture can be used.

c) Instrument calibration verification must be performed:

(1) at the beginning and end of each analytical batch (however, if an internal standard is used, only one verification needs be performed at the beginning of the analytical batch),

(2) whenever it is suspected that the analytical system may be out of calibration or might not meet the verification acceptance criteria,

(3) if the time period for calibration or the most previous calibration verification has expired, or

(4) for analytical systems that contain a calibration verification requirement based on the number of runs, the number of runs is exceeded.

d) Results of the calibration verification must be documented and retained by the laboratory and be available for review. Sufficient raw data records shall be retained to permit reconstruction of the calibration verification (e.g., verification date and time, test method SOP used, instrument identifier,

each analyte name, analyst's name, concentrations used and instrument responses obtained, degree to which results matched calibration curve or response factor; and any equations or coefficients used to convert instrument responses to concentration). Calibration verification records must explicitly connect the verification data to the calibration (i.e. verification was performed on the same instrument and the most recent calibration was verified).

e) Criteria for the acceptance of a calibration verification must be established (e.g., relative percent difference from calibration).

f) If the calibration verification results are outside of acceptance criteria, corrective actions must be taken. Following completion of corrective actions, two immediately consecutive calibration verifications must be analyzed. If the two consecutive calibration verifications do not yield acceptable results, then the laboratory shall recalibrate the analytical system.

g) If the laboratory has not verified calibration, sample analyses shall not occur until the analytical system is calibrated or calibration is verified, with the exception that results associated with an unacceptable calibration verification may be reported as qualified data under the following special conditions:

(1) When the acceptance criteria for the calibration verification are exceeded high (i.e., high bias) and the analyte in the associated samples is not detected, then the non-detect may be reported. Otherwise, the samples affected by the unacceptable calibration verification shall be reanalyzed after the analytical system has been calibrated or calibration has been verified.

(2) When the acceptance criteria for the calibration verification are exceeded low (i.e., low bias) and the concentration or amount of the analyte in the associated samples exceeds a regulatory limit or decision level, the concentration or amount may be reported. Otherwise, the samples affected by the unacceptable verification shall be reanalyzed after the analytical system has been calibrated or calibration has been verified.

5.5.9.2 Essential Quality Control Procedures

In addition to the requirement for evaluation, the following general quality control procedures shall apply, wherever applicable. The manner in which they are implemented is dependent on the types of tests performed by the laboratory (i.e., chemical, whole effluent toxicity, microbiological, radiological, air) and is further described in Appendix D. The standards for any given test type shall assure that the applicable principles are addressed:

a) The laboratory shall have quality control procedures in place to monitor the performance of the measurement system on an on-going basis, including:

(1) procedures to ensure that the measurement system is free of laboratory induced

interferences;

(2) procedures to identify if and when analytical instruments are in an out-of-control condition;

(3) procedures to verify continuing analyst proficiency;

(4) procedures to ensure the suitability of reagents and standards; and

(5) measures such as temperature, humidity, light, or specific instrumental conditions, to assure constant and consistent test conditions (both instrumental and environmental) where required by the test method.

b) All quality control measures shall be assessed and evaluated on an on-going basis, and quality control acceptance criteria shall be used to determine the usability of the data. (See Appendix D.)

c) The laboratory shall have procedures for the development of acceptance /rejection criteria where no method or regulatory criteria exist. (See Section 5.5.8.1, Sample Acceptance Policy.)

The essential quality control measures for testing are found in Appendix D of this Chapter.

To the extent possible, samples shall be reported only if all quality control measures are acceptable. If a quality control measure is found to be out of control and the data is to be reported, all samples associated with the failed quality control measure shall be reported with the appropriate data qualifier(s).

5.10.10 Documentation

For all environmental testing studies, the documentation of the results of the Method Evaluation and Ongoing Quality Control evaluation elements (see Table A) shall be reported to the client along with the actual test results.

Table A

Evaluation Element	Method Evaluation	Quality Control
Calibration	Initial calibration	Daily verification
Detection Limit	Initial determination for each SOP on each matrix	Whenever analytes are to be reported at DL
Quantitation Limit	Initial determination for each SOP on each matrix	Matrix spike at QL for each batch
Bias	Initial determination for each SOP on each matrix at mid-point of range	Matrix spike/matrix spike duplicates for each batch at decision level
Precision	Determine for each SOP on each matrix at mid-range	Matrix spike/matrix spike duplicates at decision level, or multiple samples for each batch
Selectivity	Determine for each SOP	Confirm analyte identity for each positive hit on an analyte
Analytical System Performance	see above	Laboratory Control Sample
System Cleanliness	see above	Field blank System blank
Analyst Proficiency	see above	Performance Test Sample